REMARKS

The presently claimed invention concerns a method for biasing the amplification of the DNA molecules in a sample such that a nucleic acid molecule having a specific nucleotide at a selected position (e.g., a polymorphic site) is preferentially amplified relative to an otherwise identical nucleic acid molecule not having the specific nucleotide at the selected position. The method is useful for preferentially amplifying at least a portion of one allele of a gene relative to another, different allele of the gene in a sample containing both alleles of the gene as well as for other purposes.

It is often desirable to obtain a relatively pure sample of one allele of a gene. For example, where an individual harbors two different alleles of a gene of interest, haplotyping the gene is practical only if one can obtain a relatively pure sample of one of the two different alleles. The method of the invention provides a means for obtaining a relatively pure sample of one of two different alleles of a gene in a DNA sample containing both alleles through the use of a so-called biased amplification reaction that greatly favors amplification of one of the two alleles present in the sample. For example, the method of the invention can be used to preferentially amplify the maternal allele of a gene of interest relative to the paternal allele where the two alleles differ in sequence at a polymorphic site. Thus, the methods of the invention can be used to obtain a relatively pure sample of either allele for use in haplotyping.

The method of the invention relies, in part, on the fact that the presence of a stable stem-loop structure in a nucleic acid molecule can inhibit amplification (e.g., PCR amplification) of the molecule if the stem-loop is relatively stable.

To illustrate the claimed methods, consider a subject that is known or thought to be heterozygous at a particular polymorphic site. For example, at the polymorphic site, there can be either a "T" or a "C" on the minus strand and it is considered desirable to haplotype the patient by obtaining a relatively pure sample of the "C" allele. This can be achieved by using the presently claimed methods. In this instance specially designed primers that permit greater amplification of the "C" allele compared to the "T" allele are used as follows.

A sample of DNA taken from the patient is subjected to PCR amplification using two primers. The first primer binds to the plus strand and is a conventional primer. The second primer binds to the minus strand and is designed to incorporate sequences into the amplification product that cause the formation of a perfect (relatively stable) stem-loop structure upon amplification of one allele, in this case the "T" allele, and forms an imperfect (relatively unstable) stem-loop structure upon amplification of the other allele, in this case the "C" allele.

A portion of the second primer is complementary to the minus strand of both the "T" allele and the "C" allele. A 5' portion of this primer is not complementary to the minus strand of either allele, although after two rounds of amplification, this portion will be incorporated into the amplification product. This 5' portion that is not complementary to the minus strand includes a sequence that will, during amplification, cause the incorporation into the minus strand of a sequence that can base-pair with the region surrounding the polymorphic site thus forming the stem of a stem-loop. It is important to realize that the 5' portion of the primer itself does not hybridize to the region surrounding the polymorphic site. Rather it leads to the incorporation into the amplification product of a sequence that can base-pair with the region surrounding the polymorphic site. Thus, the primer includes a 5' region that is the reverse sequence of the sequence surrounding the polymorphic site. Put differently, if the sequence at the polymorphic site is 5'-AGGTCTA-3' on the strand to which the primer hybridizes, the sequence at the 5' end of the primer would be, e.g., 5'-ATCTGGA-3' and the sequence incorporated into the amplification product would be 5'-TAGACCT-3', allowing it to base-pair with the region containing the polymorphic site.

After two rounds of amplification, the 5' portion of the second primer, indeed the entire primer, has become incorporated into the amplification product causing the minus strand to include the complement of the primer sequence. As discussed above, the sequence incorporated by the 5' portion of the primer is perfectly complementary to the region surrounding the polymorphic site. Thus, to achieve amplification of the "C" allele while inhibiting amplification of the "T" allele, the incorporated sequence includes an "A" that can base-pair to the "T" at the polymorphic site. During the next round of denaturation and annealing, the minus strand of the amplification product forms a stem-loop structure having a perfectly matched stem—if a "T" is present at the polymorphic site. This stem includes the polymorphic site "T" base-paired to the

"A" in the incorporated sequence. The stem-loop structure is stable enough to inhibit further amplification of the "T" allele relative to the "C" allele.

The situation is different for the C allele. Again, after two rounds of amplification, the complement of the complement of the second primer is incorporated into the amplification product. Of course, in this case, the incorporated 5' portion is not perfectly complementary to the region surrounding the polymorphic site. This is because the primer was designed introduce a sequence perfectly complementary to the region of the polymorphic site only when a "T" is present at the polymorphic site, not when a "C" is present. Thus, upon the next round of denaturation and annealing, the stem of the stem-loop that forms is imperfect. It contains a mis-match at the polymorphic site. This stem-loop is not stable enough to significantly inhibit further amplification relative to a molecule having a perfect stem-loop. As a result, "C" allele nucleic acid molecules undergo additional amplification while amplification of "T" allele nucleic acid molecules is inhibited.

Power of Attorney and Mailing Address

The final Office Action was mailed to Wesley B. Ames at Brobeck, Phelger & Harrison LLP. Applicant notes that the power of attorney in this matter is now in the undersigned and requests that all mail be directed to the undersigned. A copy of the Combined Declaration and Power of Attorney filed May 30, 2001 is enclosed for the Examiner's reference.

Rejections Under 35 U.S.C. §102(e)

The Examiner rejected claims 10-16 as allegedly anticipated by Tygai et al. (U.S. Patent No. 6,277,607; "Tygai"). According to the Examiner, Tygai teaches "a method for amplification of a DNA molecule wherein a first nucleic acid molecule having a mutated nucleotide at the polymorphic site is amplified to a greater extent than a second nucleic acid having a different nucleotide at the said polymorphic site." The Examiner goes on to argue that Tygai discloses "contacting a sample of DNA with a pair of primers, one of which is complementary to a nucleic acid strand and the other of which is complementary to the other strand, and amplifying the nucleic acid, thereby the polymorphic site with mutated nucleotide is amplified to a greater extend [sic] than the second o other nucleic acid molecule with a different nucleotide at the said

site.... Upon amplification the said primers form a stem-loop structure...." Finally, the Examiner concludes the "disclosure of Tygai et al. meets the limitations in the instant claims." Applicant disagrees and respectfully traverses this rejection

The present claims are not anticipated by Tygai. Indeed, the method disclosed by Tygai is very different from that of the present claims. While it is true that Tygai's method and the currently claimed method are both intended to achieve differential amplification of two nucleic acid molecules that are nearly identical, e.g., two molecules that differ at only a single nucleotide, the methods themselves are very different in overall concept and in the specific steps carried out and primers used.

Tygai employs a primer that hybridizes to the polymorphic site while the primers of the claimed method flank the polymorphic site

Tygai attempts to achieve selective amplification using a primer that forms a hairpin comprising a loop and a stem (col. 5, lines 9-11). The sequence within the loop is completely complementary to one, but not the other, of the two different nucleic acid molecules. Differential amplification is achieved by the fact that the hairpin primer binds more efficiently to one of the two different nucleic acid molecules than to the other. As Tygai explains, "[i]f the sample contains a nucleic acid differing from target 5 by a nucleotide that is not complementary to the sequence in loop 3, the loop cannot bind to that nucleic acid and 3' arm 4 cannot anneal to the nucleic acid and initiate DNA synthesis" (col. 5, lines 47-51). Thus, to the extent that Tygai achieves differential amplification, it is because the hairpin primer hybridizes differentially to the two different nucleic acid molecules.

The method of the present invention achieves differential amplification in an entirely different manner. The primers are <u>not</u> designed to hybridize differentially to the two different nucleic acid molecules as in the method of Tygai. Indeed, the primers of the presently claimed method are designed to <u>flank</u> the polymorphic site, not hybridize to it. Thus, claim 1 specifies that the first and second primers "hybridize to both the first and the second nucleic acid molecule at locations which flank the polymorphic site" Nothing in Tygai suggest the sue of primer that flank the polymorphic site. Indeed, the method of Tygai <u>requires</u> that one of the primers hybridizes to the polymorphic site. If it did not, differential amplification could not occur. For

this reason alone, it is clear that Tygai's method differs from the presently claimed methods and Tygai cannot anticipate the presently claimed invention.

In the Claimed Method Incorporation of Primer Sequence Enhances Differential Amplification; in Tygai's Method Incorporation of Primer Sequences Can Reduce Differential Amplification

How very different the method of Tygai is from the presently claimed method becomes even clearer when one considers the impact of the incorporation of primer sequences into the amplification products. In the case of the present claims, incorporation of the primer sequences into the amplification product is the very means means by which differential amplification is a achieved. In sharp contrast, incorporation of primer sequences into the amplification product in the method of Tygai can actually reduce differential amplification.

As explained above and as specified in the current claims, in the current method incorporation of a 5' portion of one of the primers into the amplification product "will upon further amplification yield products what form a stable stem-loop structure, which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site". It is this formation of a perfectly matched stable stem-loop structure that causes differential amplification to occur since those molecules having a perfectly matched stable stem-loop are not amplified as readily as those having no stem-loop or an imperfectly matched stem loop. In contrast, Tygai explains that incorporation of primer sequences into the amplification product, far from enhancing differential amplification, can actually reduce differential amplification.

> It will be appreciated that, even when the 5' arm [of the primer] is completely non-complementary to the intended target, amplicons synthesized by copying the oligonucleotides containing a primer will contain complete complements of the primer. This may reduce the discriminatory effectiveness, including the suppression of false amplicons, achieved in the first round of synthesis.

(col. 6, liens 12-18) This difference in the impact of incorporated primer sequences makes it very clear that the currently claimed method and the method of Tygai is a clear example of the very significant differences between the present claims and the cited art.

In sum, Tygai neither teaches nor suggests the use of primers which flank a polymorphic site as required by the present claims. Moreover, Tygai neither teaches nor suggests the use of a primer which includes "a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products what form a stable stem-loop structure, which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site" Thus, Tygai does not disclose at least two different elements of the present claims. "Anticipation under 35 U.S.C. §102 requires the disclosure in a single piece of prior art of each and every limitation of a claimed invention." *Apple Computer, Inc. v. Articulate Systems, Inc.*, 234 F.3d 14 (Fed. Cir. 2000). The cited prior art fails to teach each and every limitation of the present claims, and Applicant respectfully requests that the rejections under 35 U.S.C. §102(e) be withdrawn.

Conclusion

A Request for Continued Examination is being filed with this amendment in order to cite new art in this application. Also enclosed are papers relating to a correction of inventorship in this application.

Applicant asks that all claims be allowed. Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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